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Structural Transition of Actin Filament in a Cell-Sized Water Droplet

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アクチンフィラメントは細胞の形状・強度・運動に関わる負に帯電した剛直荷電高分子であり、細胞中に多く分布し様々な形態をとっている。細胞膜の内膜に多いポスファチジルエタノールアミン（PE）やホスファチジルセリン（PS）などのリン脂質が構成する膜表面とアクチンフィラメントとの相互作用を調べるため、内部にアクチンフィラメントを封入したマイクロメートルスケールの油中水滴を作製し油水界面に脂質膜を形成させ、多価カチオン存在下での脂質とアクチンフィラメントの凝集構造を調べた。

Actin filament is the constituent of cytoskelton and play an important role in controlling the cell shape. Actin is a semiflexible polymer with negative charge. Actin filament forms a variety of assembly in cytopasm, especially in the vicinity of membrane. It was shown that in giant liposome actin filament attaches onto the surface of membrane.[1] In biomembranes, PC distributes for the most part in the outer membrane, and phosphatidylethanolamine(PE) and phospatidylserine(PS) have high proportion on inner membrane. It is expected that the asymmetry of membrane is important for cell function. It was also reported that dynamics of PE orientation play a role for the absorption-desorption of actin filament to membrane during cell division.[2] However, the methodology to prepare such giant liposome has not been established yet. In order to prepare stable giant liposome we must use in large part PC for lipid of which giant liposome is composed. In the present study, we use micro-scale Water Droplet in Oil (WDO). We consider the water droplet of WDO as a model of cytoplasm, where the head of lipid in water-oil interface direct to the water phase. To investigate interaction between actin filament and model membrane composed of PE and PS, we conducted a microscopic observation of WDO encapsulating actin filaments.

To obtain WDO encapsulating actin filament, 1 μ l F-buffer (2 mM Tris/HCl pH 7.5, 0.2 mM $CaCl_2$, 2 mM $MgCl_2$, 0.2 mM dithiothretol, 0.005% NaN3) is added to 200 μ l mineral oil dissolving 1mM lipid, then 1 μ l G-actin is added to that droplet and quickly mix round. The size of WDO is dependent on time of the preparation and also on the intensity of voltex mixing. Under there conditions, actin filament is formed in WDO within two hour (Fig.1).

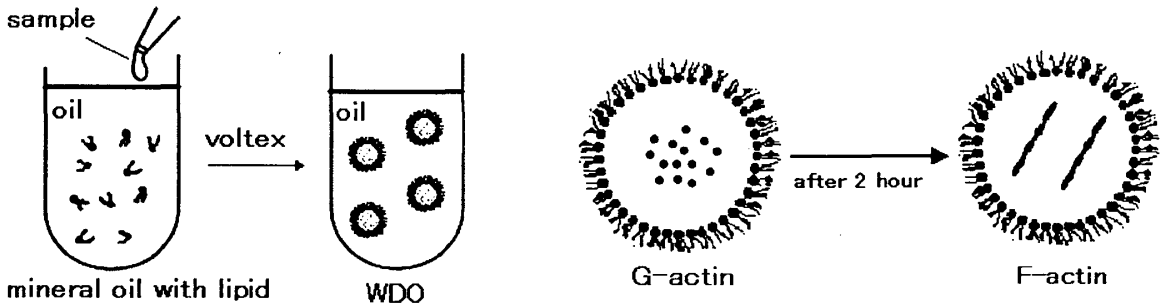

Fig.1: Experimental procedure to obtain water-droplets, WDO, encapsulating actin.

It is found that PE (neutral lipid) membrane attaches actin filaments, while in the case of PS (negative lipid) membrane actin filaments distribute uniformly in the water phase without adsorption to membrane surface (Fig.2). It is expected that PE exhibits positive charge in the presence of bivalent cation such as Mg^{2+} and Ca^{2+},[3] causing the attractive interaction between the membrane surface and actin filaments. While PS membrane having negative charge does not absorb actin filament.

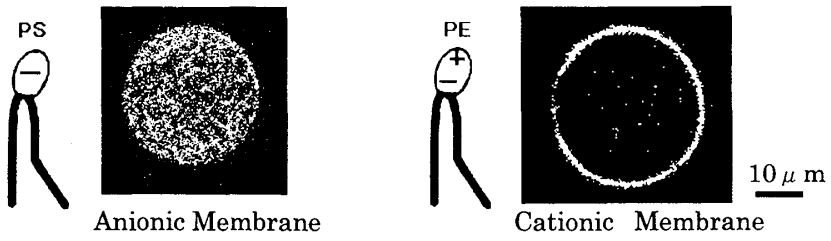

Fig.2: Confocal image of WDO with PS and PE layers, encapsulating actin filament

Actin filaments undergo bundle transition at 12mM Mg^{2+} in a discrete manner, as shown in Fig.3. In WDO with PS membrane, actin filament attaches to membrane surface at the Mg^{2+} concentration less than 12mM(Fig.4). It is expected that the assembling transition of actin filament is first-order transition and membrane has lower remaining charge than actin filament because of geometrical figure, then membrane absorbs actin filament at Mg^{2+} concentration less than 12 mM.

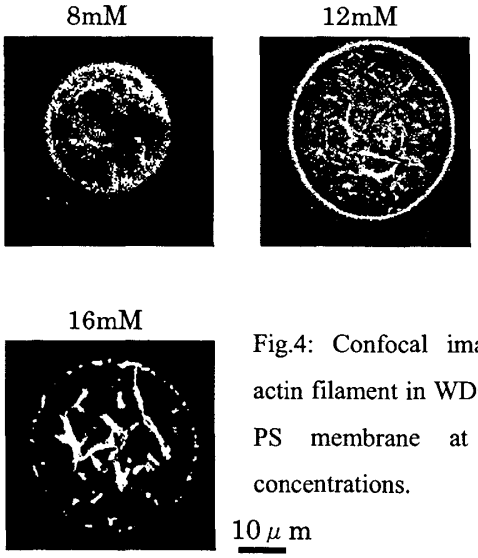

Fig.4: Confocal image of actin filament in WDO with PS membrane at Mg^{2+} concentrations.

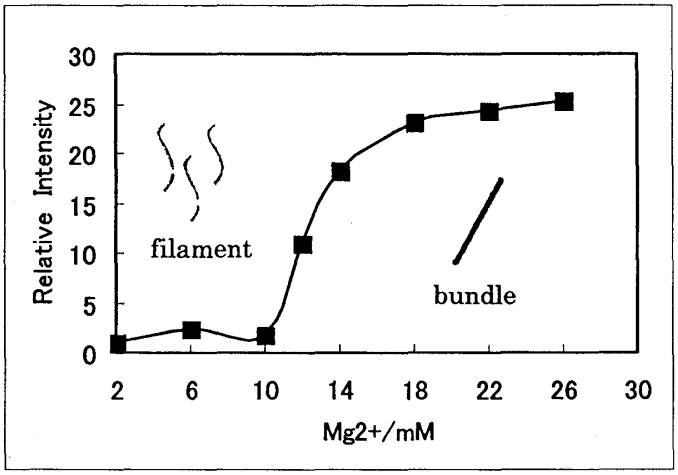

Fig.3: Formation of actin bundle as monitored by light scattering.

[1] L. Limozin, M. Bärmann, E. Sackmann, Eur. Phys. J. E. 10 (2003) 319
[2] K. Emoto, T. Kobayashi, Proc. Natl. Acad. Sci. USA. 93 (1996) 12867
[3] C. Gicquaud, P. Wong, Biochem. J. 303 (1994) 769